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Factors affecting the overwintering of tadpoles in a temperate amphibian

Patrick T. Walsh[1]*, J. Roger Downie[2] & Pat Monaghan[2]

[1] Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3JT

[2] School of Life Sciences, College of Medical, Veterinary & Life Sciences, Graham Kerr Building, University of Glasgow, Glasgow G12 8QQ.

Short title: Effect of environmental conditions on larval overwintering

*Corresponding Author: Patrick T Walsh, University of Edinburgh, UK;
Email: Patrick.Walsh@ed.ac.uk
Phone: 44 (0)131 650 5474
Fax: 44 (0)131 650 5455

Abstract

Temperate anuran amphibians generally complete metamorphosis by the autumn of their natal year, but there are an increasing number of reports of overwintering of the larval tadpole phase. We currently know little about the environmental cues that trigger this change in the temporal pattern of development, or if it has any fitness consequences. In an experimental study in the laboratory, we examined the effects of environmental temperature and food availability on the temporal pattern of larval development and the proportion of individuals that overwintered as larvae, in *Rana temporaria* from the UK. The proportion of overwintering tadpoles increased with lower temperature and food availability. No overwintering tadpoles appeared to arrest their larval development early in the season, contrary to expectations from field observations. Therefore, other factors beyond mean temperature and food availability may be involved in the overwintering phenomenon observed in the field. Furthermore, all overwintering tadpoles were large enough to commence metamorphosis in the autumn, but this was delayed until spring, and on completion of metamorphosis overwintered tadpoles were larger than individuals that had completed metamorphosis within their first summer. While developmental and growth constraints certainly play a role in overwintering as larvae in the laboratory, the larger size on completion of metamorphosis may represent an adaptive advantage for this phenomenon. The extent to which the larger size of overwintering tadpoles is advantageous would presumably vary with local conditions and individual survival prospects.

Key words: development, environmental temperature, food availability, metamorphosis, phenology, phenotypic plasticity, overwintering, *Rana temporaria*

Introduction

In many temperate organisms, life cycles and life history events are linked to seasonal environmental changes (Foster & Kreitzman, 2009). Among temperate amphibians the causes and consequences of events timed in association with spring are well studied (e.g. date of first appearance: Beebee, 1995; Blaustein *et al.*, 2001; date of first calling: Gibbs & Breisch, 2001; Lovett, 2013; date of first spawning: Beebee, 1995; Reading & Clarke, 1999; Blaustein *et al.*, 2001). However, the timing of events coincident with the onset of winter, particularly an individual's first winter, while likely to be important (Hurst & Conover, 1998; Bale & Hayward, 2010), have received less attention.

In most temperate anuran amphibians, the larval stage is completed before the onset of winter (Stebbins & Cohen, 1995) and larval individuals are absent from the aquatic environment from autumn until the following spring when new egg clutches are deposited. In these temperate amphibians, freezing winter temperatures and seasonal drying are thought to limit the duration of the aquatic larval stage (Wilbur & Collins, 1973). However, there is growing evidence that, in temperate regions, overwintering can occur as tadpoles (Berven, Gill, & Smith-Gill, 1979; Collins & Lewis, 1979; Collins, 1979; Archibald & Downie, 1996; Pintar, 2000; Gollmann, Baumgartner, & Gollmann, 2001; Fellers *et al.*, 2001; Lai *et al.*, 2002; Snodgrass *et al.*, 2005; Lauck, Swain, & Barmuta, 2005; Bland, 2008; Walsh, Downie, & Monaghan, 2008a; Hsu, Kam, & Fellers, 2012; Fominykh & Lyapkov, 2012; Corbalán *et al.*, 2014) and, at least in some species, the phenomenon may be wide-spread (e.g. *Rana temporaria*: Archibald & Downie, 1996; Pintar, 2000; Bland, 2008; Walsh *et al.*, 2008a).

Thus far there has been little effort to determine the factors that favour larval overwintering, at least in temperate amphibian species which could face harsh, potentially fatal, winter conditions. Temperature is known to affect larval duration (Berven *et al.*, 1979) and has also been proposed to play a role in overwintering (Collins, 1979; Lai *et al.*, 2002).

However, temperatures simulating field conditions at a single site where larvae overwinter did not induce overwintering in the laboratory in the temperate frog *Rana temporaria* (Walsh *et al.*, 2008a). Furthermore, fixed temperatures, which are commonly used in larval duration studies, do not accurately reflect the realities of larval development in temperate species, where temperatures can vary considerable both seasonally and inter-annually.

The impact of food availability or competition level on overwintering is even more equivocal. Amphibian larval development could be prolonged if resources are low, constraining development (Leips & Travis, 1994; Audo *et al.*, 1995; Hsu *et al.*, 2012); conversely, abundant resources can extend the larval phase so that individuals can take advantage of good conditions (Wilbur & Collins, 1973; Werner, 1986; Harris, 1987; Audo *et al.*, 1995; Shafiei, Moczek, & Nijhout, 2001; Doughty & Roberts, 2003). However, even where high and low resources have been reported to extend the larval period, this rarely includes delaying metamorphosis beyond winter in temperate climates. Yet, both high (Freeman & Bruce, 2001) and low food availability (Pintar, 2000; Hsu *et al.*, 2012) have been suggested as causes of larval overwintering, potentially depending on when the heightened food or deprivation conditions occur (Audo *et al.*, 1995).

To begin to understand the factors that might trigger tadpole overwintering, particularly those that do so facultatively (e.g. Walsh *et al.*, 2008a), we manipulated temperature and food availability in the laboratory to test whether changes in these parameters can induce larval overwintering in the common frog *Rana temporaria*. We also sought to follow any larvae, induced to overwinter, through to metamorphosis to determine whether overwintering as larvae confers a size advantage.

R. temporaria is a medium sized anuran (adult size: up to 9 cm) found throughout Europe, from northern Spain to Scandinavia. It is an explosive breeder, with a breeding season in Britain generally lasting only two weeks (Beebee & Griffiths, 2000), that can

commence between early January and early April depending on conditions. Larvae hatch from eggs approximately 10 – 14 days after being deposited, depending on temperature, and become free-swimming tadpoles a few days later. Development and metamorphosis into juveniles generally takes place between 10 and 15 weeks later (Beebee & Griffiths, 2000). Overwintering of *R. temporaria* as larvae has been reported in the UK (Archibald & Downie, 1996), and appears facultative, with significant numbers of individuals persisting in ponds until the subsequent spring (Walsh *et al.*, 2008a). It is thought not to occur across Scandinavia (Koskela, 1973; Miaud, Guyetant, & Elmberg, 1999) and reports of larval overwintering in Austria are disputed (Pintar, 2000, contested by Gollmann *et al.*, 2001).

Methods and Materials

This study was run in parallel with Walsh *et al.* (2008a) and followed the same methodology for the collection and rearing of spawn. Six egg clumps of *Rana temporaria* were collected and brought into the laboratory in March 2005 from two sites in Glasgow, Scotland (Robroyston Marsh: 55.89° N, 4.19° W, *c.* 81 m.a.s.l.; Queens Park: 55.83° N, 4.27° W, *c.* 28 m.a.s.l.). The collection sites were chosen because they were not part of a broader field investigation of larval overwintering, so there was no information on the incidence of overwintering in the source ponds. However, they are located near other ponds where over-wintering of *R. temporaria* tadpoles has been observed (Walsh, PT, unpublished data).

When the tadpoles were free-swimming (approximately Gosner (1960) stage 25), seventy-five tadpoles were transferred to each of 24 experimental tanks, measuring 30 x 20 x 20 cm, filled with 11 L of de-chlorinated, copper-free, aerated water and maintained within the laboratory at 5.5°C. Tadpoles were randomly allocated to the experimental tanks from the different egg clumps to ensure no differences in genetic variability between tanks or treatments. Ambient photoperiod, tank cleaning and monitoring were the same as in Walsh *et*

al. (2008a). Three temperature regimes with two food availability levels at each temperature were established, giving six treatments with four tanks per treatment.

From mid-April, the water temperature was changed on a monthly basis as shown in Figure 1 to create three temperature regimes. The regimes simulate natural environmental temperature conditions throughout the season in ponds inhabited by *Rana temporaria* across Scotland (Walsh *et al.*, 2008a; Muir *et al.*, 2014). Each regime had a different monthly increase in temperature, so that the peak temperatures in August were as follows: 1) high – peaked at 18°C; 2) medium – peaked at 14°C; and 3) low – peaked at 10°C, and thereafter a monthly decrease in temperature back to 5°C in November (Fig. 1). The low temperature treatment regime is characteristic of high elevation, high latitude sites; and the high temperature treatment characteristic of low elevation, low latitude sites in Scotland. Feeding treatments were established using the same methodology presented in Walsh *et al.* (2008a).

Once a week ten individuals from each tank, selected at random, were measured and then returned to their tanks. Wet mass was measured using an electronic balance (± 0.1 mg) and snout-vent length (SVL) was measured with dial callipers (± 0.1 mm). Development was determined, at x 10 magnification, according to Gosner's (1960) staging table. Tanks were checked weekly for individuals beginning metamorphic climax, indicated by the emergence of the forelimbs (Gosner stage 42; Walsh, 2010); these were removed and housed individually until the completion of metamorphosis in small plastic trays with ambient photoperiod and at 14°C. The trays had sloped bottoms providing a small pool of water at one end and dry ground at the other. Mass and SVL measurements were taken from a random subsample from each treatment group on the completion of metamorphosis, and then individuals were released at the site where spawn was collected.

Individuals that did not commence metamorphosis by mid-November were considered to be overwintering and were then maintained at 5°C and fed, as above, until the

following spring (March) when the temperature was increased to 14°C over the course of a week and metamorphosis took place under the same conditions as individuals that completed metamorphosis prior to the onset of winter. On completion of metamorphosis, mass and SVL measurements were taken as before.

Statistical Analysis

All values are given as means ± SE. Survival and overwintering were analysed using GLM on arcsine-transformed percentages. Where tank mortality was 100%, which only occurred within the low temperature treatment, the percentage of overwintering individuals could not be calculated and was omitted from overwintering analysis. Comparisons of the size and stage of overwintering tadpoles were made using GLMM, with tank as a random factor to control for the possibility of any tank effects. Comparisons between the size of individuals from the medium temperature treatment completing metamorphosis in August and those that metamorphosed after overwintering were made using t-tests. Developmental rate, taken as the developmental stage reached by week 11 of the experiment, was analysed using GLMM, with tank as a random factor. Week 11 was selected because metamorphosis was first observed in week 12. The daily rate of development was calculated between each incremental increase in temperature through the season, by taking the mean Gosner (1960) stage for each tank at the last measurement prior to an incremental increase and the final measurement before the subsequent incremental increase, as indicated in Fig. 1. This was analysed using GLMM, with tank as a random factor and the incremental periods as a repeated measure. Post-hoc comparisons were made using Bonferroni-corrected pairwise comparisons of main effects.

Results

Survival and overwintering

Higher temperature resulted in higher survival through to metamorphosis or the specified overwintering date ($F_{2,18}$ = 187.24, P < 0.001) but survival was not affected by food availability ($F_{1,18}$ = 0.01, P = 0.942; Table 1). Of these tadpoles that survived, the proportion overwintering was negatively affected by food level and temperature: thus, as temperature ($F_{2,13}$ = 374.59, P < 0.001) and available food ($F_{1,13}$ = 12.42, P = 0.004) decreased the proportion of overwintering individuals increased (Table 1). The high food/high temperature treatment had the highest survival rate, and all individuals metamorphosed before November. No individuals survived in the high food/low temperature treatment, but all surviving individuals in the low food/low temperature treatment overwintered. In the medium temperature treatment, overwintering occurred in both food treatments but was lower in the high food treatment.

Overwintering individuals

Overwintering tadpoles did so at a relatively late developmental stage (overall mean stage: 38.2 ± 0.5 SE), and the stage they had reached did not differ across the three temperature regimes ($F_{2,5.34}$ = 0.956, P = 0.442; Table 1). Stage 38 is characterized by relatively well-developed hind limbs with all toes differentiated, and is only three developmental stages before the onset of metamorphic climax.

Overwintering individuals differed in mass ($F_{2,17}$ = 8.54, P < 0.005) and SVL ($F_{2,17}$ = 11.08, P < 0.005) among the temperature treatments. This is due to the individuals that overwintered in the high temperature treatment being extremely small, less than 30% of the size of the other two temperature treatments (mass: both Post-hoc test P < 0.05; SVL: both Post-hoc test P < 0.005). There was no difference in mass between the overwintering

individuals in the medium and low temperature treatments (543.4 ± 31.6 mg; Post-hoc test $P = 0.63$) and SVL (13.8 ± 0.2 mm; Post hoc test $P = 0.92$).

Individuals in the medium temperature treatment that were still tadpoles at the onset of winter were heavier, but no different in length, than individuals that started to metamorphose (i.e. reached stage 42) during the summer and autumn (Fig. 2).

Winter survival of larvae was relatively high in the low and medium temperature treatments, with all nine of the low temperature treatment individuals and eight of the eleven medium temperature treatment individuals surviving until the following spring. Neither of the two high temperature treatment individuals survived until the spring.

Individuals from the medium temperature treatment that overwintered as larvae and metamorphosed in the subsequent spring were 30.8% heavier and 10.1% longer than those from the same treatment that metamorphosed in the summer and autumn (Fig. 3).

Development rate and body mass

Development rate, measured as the Gosner stage reached by week 11, was fastest in the high temperature treatment. The medium and low temperature treatments progressed 20.0% and 40.4% slower, respectively ($F_{2,23.66} = 62.20$, $P < 0.001$, Fig. 4). Higher food availability resulted in individuals reaching a more advanced developmental stage by week 11 ($F_{1,72.79} = 15.32$, $P < 0.001$, Fig. 4) and this was consistent across temperature regimes ($F_{1,23.66} = 0.83$, $P = 0.450$).

Development progressed continuously in all treatment groups, with no apparent arrest in development during the simulated summer (Fig. 4). The daily rate of development accelerated through the season, as the temperature was increased according to Figure 1 ($F_{2,59} = 31.20$, $P < 0.0001$; Fig. 5). The rate was not significantly different between the first two

temperature increases (Post hoc test $P = 1.00$), but was much quicker during the third (Post hoc test $P < 0.0001$).

Body mass in week 6, the last week where there were at least ten tadpoles per tank, decreased with decreasing temperatures ($F_{2,239} = 35.58$, $P < 0.001$) and decreasing food availability ($F_{1,239} = 80.03$, $P < 0.001$; Fig. 6). However, there was no difference in mass between the high and low food treatment at low temperatures (Fig. 6; Post hoc test $P = 0.46$).

Discussion

Larval overwintering was induced in *Rana temporaria* under laboratory conditions for the first time, replicating the observation of larvae of this species present in ponds in the field during winter (Archibald & Downie, 1996; Bland, 2008; Walsh *et al.*, 2008a). This indicates that this phenomenon is amenable to further study under laboratory conditions. The proportion of *Rana temporaria* individuals overwintering as larvae in the laboratory was affected by environmental conditions manipulated in this study: temperature and food availability. Lower temperatures and reduced food availability experienced during the summer season resulted in a higher proportion of the surviving individuals remaining as tadpoles during the winter. This provides support for the hypotheses that lower temperatures (Collins, 1979; Lai *et al.*, 2002) and reduced food (Hsu *et al.*, 2012) contribute to larval overwintering, though these factors are not the whole story as is often assumed.

The observed effects of temperature and food availability, under laboratory conditions, appear to eliminate the prospect that larvae overwinter to take advantage of beneficial conditions in the aquatic environment (Wilbur & Collins, 1973; Werner, 1986). However, there still remain two possible explanations for the phenomenon; reduced temperature and/or food availability either: 1) act as a physiological constraint preventing metamorphosis from occurring within a single season; or 2) result in an adaptive decision to

delay metamorphosis to make the best of the available conditions (i.e. reflecting a trade-off: Wilbur & Collins, 1973; Travis, 1984; Werner, 1986).

In the low temperature treatment, all surviving individuals remained as larvae into the simulated winter, which strongly suggests that development was constrained, perhaps by cold temperatures interfering with endocrine function (Kollros, 1961; Frieden, Wahlborg, & Howard, 1965). However, having all individuals in a pond overwintering as larvae due to low temperatures is not consistent with reports from the field. Firstly, larval overwintering in wild *Rana temporaria* occurs facultatively: with some individuals within a pond doing so, while many others complete metamorphosis and overwinter as juveniles (Archibald & Downie, 1996; Walsh *et al.*, 2008a). Secondly, larval overwintering occurs in the field at temperatures very similar to the high temperature regime of the current study (Walsh *et al.*, 2008a). Finally, the low simulated summer treatment represents the lower extreme of temperatures experienced by British *Rana temporaria* (Muir *et al.*, 2014), and is not typical of the temperatures experienced at latitudes and elevations similar to the collection sites (PTW, unpublished data). Spawn taken from higher latitudes or altitudes may have responded differently to the low temperature regime, due to local adaptation (Laugen *et al.*, 2003; Muir *et al.*, 2014). These issues suggest that temperature constraints, while clearly capable of having an effect, are not likely to be functioning alone in natural occurrences of this phenomenon.

A reduced growth rate in the low food treatments may also indicate a constraint. Were overwintering the result of adaptive decisions under resource limited conditions, we might expect a trade-off, in the low food treatments, favouring development over growth to escape the poor conditions and the impending winter (Berven & Gill, 1983; Arendt, 1997). This was not observed. During the summer, tadpoles in the low food treatments were less

developed and, with the exception of the low temperature treatment, smaller than those reared with high food availability, indicating that both factors were constrained.

There are, however, complications with this interpretation. Poor pond conditions should, all else being equal, induce individuals to begin metamorphosis as soon as they have reached a minimum required size, so that they can escape those poor conditions by moving to the terrestrial environment (Wilbur & Collins, 1973; Werner, 1986). However, with the exception of the two individuals from the high temperature treatment, all overwintering individuals had reached an appropriate size to undergo metamorphosis. In fact individuals that overwintered as larvae were larger, on average, at the onset of winter than those that had reached metamorphosis in the summer and autumn. Alternatively, rather than an individual's final size, there might be a critical phase in development where a reduced growth rate will result in a delay in metamorphosis, and potentially overwintering (e.g. Thorpe, 1977; Metcalfe, Huntingford, & Thorpe, 1988; Leips & Travis, 1994). While this was not explicitly tested, it does not appear to be likely. In our study the food treatments did not change through the study and the progression of larval development accelerated with each incremental temperature increase, in all temperature treatments. Therefore, given that the individuals that did overwinter were not constrained themselves, as was found at the overall treatment level, this suggests that additional factors beyond constrained food availability are involved in the occurrence of larval overwintering.

The developmental stage and size achieved by individuals at the onset of winter may provide some insight into whether larval overwintering has adaptive value, as suggested by the larger body size, early developmental arrest and bimodal distribution in tadpole development observed in overwintering *Rana temporaria* tadpoles in the field (Walsh *et al.*, 2008a). Previous descriptive work predicted that a decision to overwinter would be reached early in the free-swimming larval phase, and development arrested at approximately Gosner

289 stages 32 – 35. After stage 35, the hind limbs, which are vital to the subsequent juvenile and
290 adult phases, begin to develop rapidly and become more vulnerable to ice damage (Lai et al.,
291 2002); they might also impair swimming performance and make individuals more susceptible
292 to predation (Brown & Taylor, 1995). However, our findings do not support the hypothesis
293 that overwintering as a tadpole occurs at an early developmental stage. Overwintering
294 occurred when the tadpoles were at a relatively late stage in larval development
295 (approximately stage 38), when hind limbs are well developed, with some individuals
296 arresting development just prior to the eruption of the forelimbs and the onset of
297 metamorphic climax (stage 41).

298 Longer development times provided by overwintering allow individuals to achieve
299 larger sizes than those that develop more quickly (Wilbur & Collins, 1973) and larger body
300 size at metamorphosis confers advantages in improved locomotion, survival and reproductive
301 fitness (e.g. Semlitsch, Scott, & Pechmann, 1988; Goater, 1994; Fischer *et al.*, 2005;
302 Chelgren *et al.*, 2006; Ficetola & De Bernardi, 2006; Walsh, Downie, & Monaghan, 2008b).
303 The larger body size of the tadpoles could also provide them with a size-refuge from
304 predators (Brodie & Formanowicz, 1983; Tejedo, 1993), reducing mortality and favouring
305 larger periods in the aquatic environment (Werner, 1986). In contrast, earlier metamorphosis
306 has been shown to be advantageous for survival to, and size at, first reproduction (Semlitsch
307 *et al.*, 1988; Govindarajulu, Altwegg, & Anholt, 2005; Schmidt, Hödl, & Schaub, 2012).
308 However, the proposed advantage of 'early' metamorphosis is an entry into the terrestrial
309 environment before other individuals metamorphose in a season, reducing competition on
310 land, and providing a longer period of terrestrial growth prior to the onset of winter
311 (Semlitsch et al 1988). Therefore, although early metamorphosis and prolonged larval
312 development appear contradictory, overwintering as larvae may actually provide advantages
313 from both longer development times and metamorphosing early in a given season. However,

the advantages of overwintering may apply differentially to particular components of the population varying with, for example, metabolic traits, anticipated final size, vulnerability to predation and competitive ability. Similarly, differences in physiology may mean that some individuals are more constrained than others.

Individuals that overwintered as tadpoles showed high levels of survival (77.3%) and were able to metamorphose the following spring, doing so earlier than 'new season' individuals would be capable of. Furthermore, on completion of metamorphosis they were heavier and longer than individuals that completed metamorphosis in the summer. While this suggests benefits of larval overwintering, data are needed on the subsequent survival, growth, and reproductive performance of individuals that overwinter as juveniles or as larvae. Unfortunately, field data on the winter survival of juveniles in the terrestrial environment is scarce, difficult to collect, and will likely be depend on the environmental context (Chelgren *et al.*, 2006).

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Tables

Table 1: Tadpole survival during the experiment for each treatment group. The number of surviving tadpoles is given as the number of individuals successfully completing metamorphosis prior to the onset of winter plus the number of pre-metamorphic tadpoles at the onset of winter. Mean per tank survival rate (± SE) as a percentage of the initial number is also given for each treatment group. The total number of individuals overwintering, mean percentage of surviving tadpoles overwintering, and mean developmental stage attained at the onset of winter in each treatment group are also given.

	Low food treatment					High food treatment				
Temperature	Survival		Overwintering			Survival		Overwintering		
treatment	n	%	n	%	Gosner stage	n	%	n	%	Gosner stage
Low	9	3.0 ± 2.6	9	100 ± 0	37.9 ± 0.4	0	0 ± 0	-	-	-
Medium	138	46.0 ± 4.7	8	5.8 ± 1.6	39.1 ± 0.8	155	51.7 ± 4.9	3	1.9 ± 0.6	38.5 ± 2.5
High	201	67.0 ± 1.8	2	1.0 ± 0.6	35.5 ± 2.5	205	68.3 ± 6.2	0	0 ± 0	-

Figure legends

Fig. 1 Water temperature throughout the season for the experimental temperature treatments

Fig. 2 Mean mass (mg) and SVL (mm) of individuals from the medium temperature treatment that remained tadpoles at the start of winter (□; n = 11) or that reached stage 42 in the summer and autumn before their first winter (■; n = 202). * t = -4.82, $P < 0.001$; NS: not significant

Fig. 3 Mean (± SE) mass (mg) and SVL (mm) of recently metamorphosed individuals that either spent winter as a tadpole and completed metamorphosis the following spring (□; n = 8) or completed metamorphosis in the summer and autumn before their first winter (■; n = 24). * t = 2.22, $P < 0.05$; ** t = 2.83, $P < 0.01$

Fig. 4 Development trajectories (as the weekly mean Gosner stage ± SE) of *Rana temporaria* tadpoles reared at low (●), medium (▲) and high (■) temperature regimes and fed on high (open shapes, broken lines) or low (solid shapes and lines) food rations from the start of the experiment until week 11 after which the first metamorphs were observed. Arrows indicate the weeks when the temperature was increased according to Fig. 1

Fig. 5 Mean (± SE) daily change in developmental (Gosner stage) across three incremental increases in environmental temperature during the first 11 weeks of the simulated summer season, when reared at low (●), medium (▲) and high (■) temperature regimes and fed on high (open shapes, broken lines) or low (solid shapes and lines) food rations. In the low temperature, high food treatment only one tank had individuals surviving into the third temperature increase, so a mean could not be taken

Fig. 6 Mean (± SE) mass (mg) of individuals at week 6 of the experiment from the three temperature treatments, provided either high (□) or low (■) food availability

Figure 1

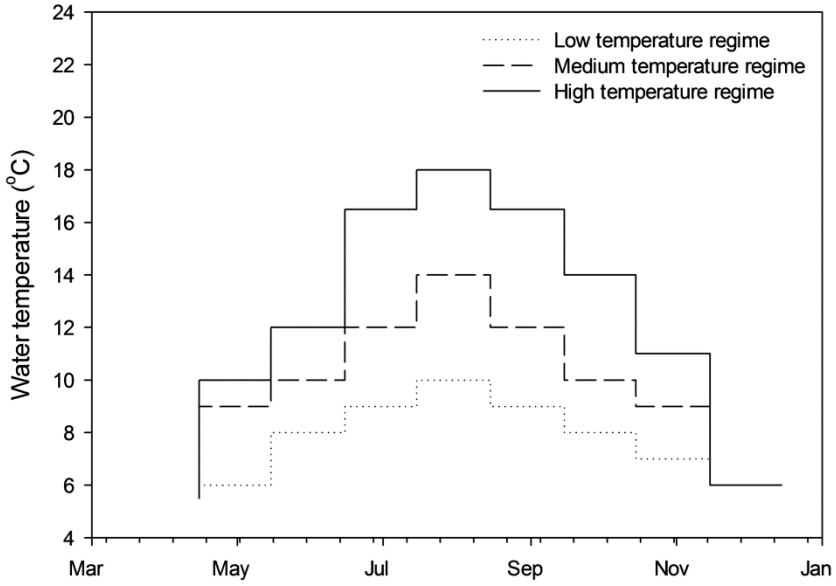

Low temperature regime
Medium temperature regime
High temperature regime
Water temperature (°C)
24
22
20
18
16
14
12
10
8
6
4
Mar
May
Jul
Sep
Nov
Jan

Figure 2

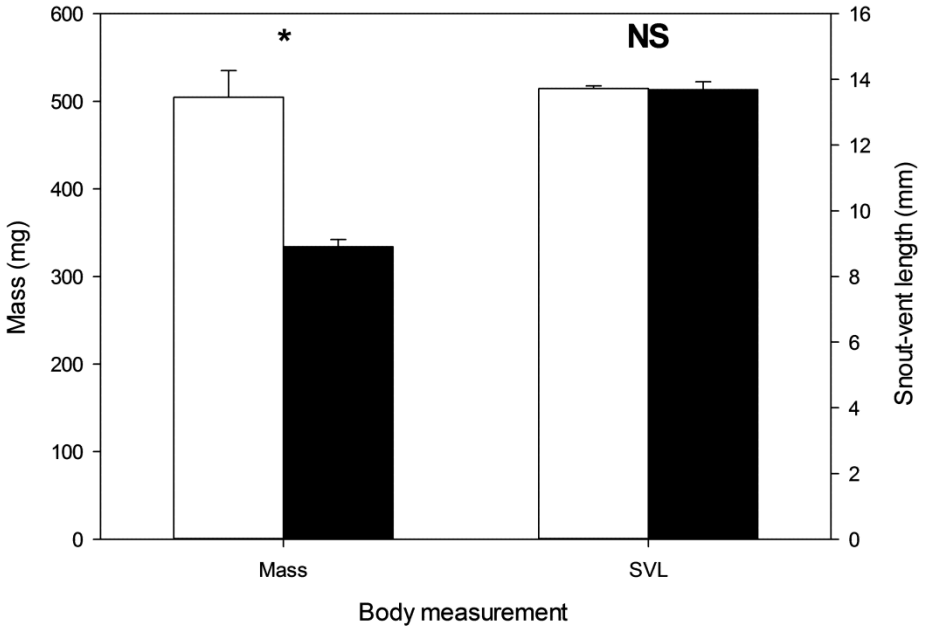

600
500
400
300
200
100
0
Mass (mg)
16
14
12
10
8
6
4
2
0
Snout-vent length (mm)
*
NS
Mass
SVL
Body measurement

Figure 3

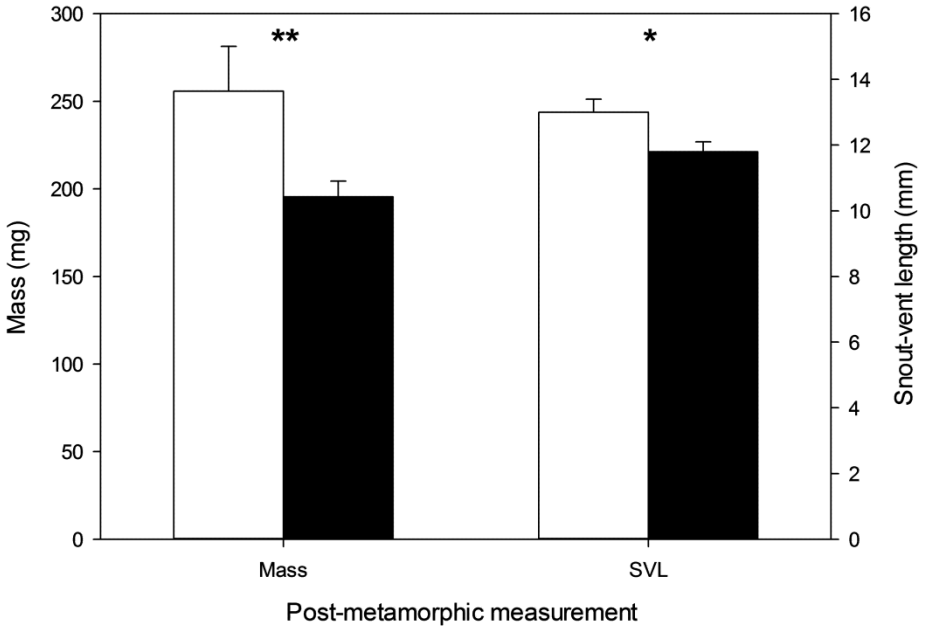

300
250
200
150
100
50
0
Mass (mg)
16
14
12
10
8
6
4
2
0
Snout-vent length (mm)
**
*
Mass
SVL
Post-metamorphic measurement

Figure 4

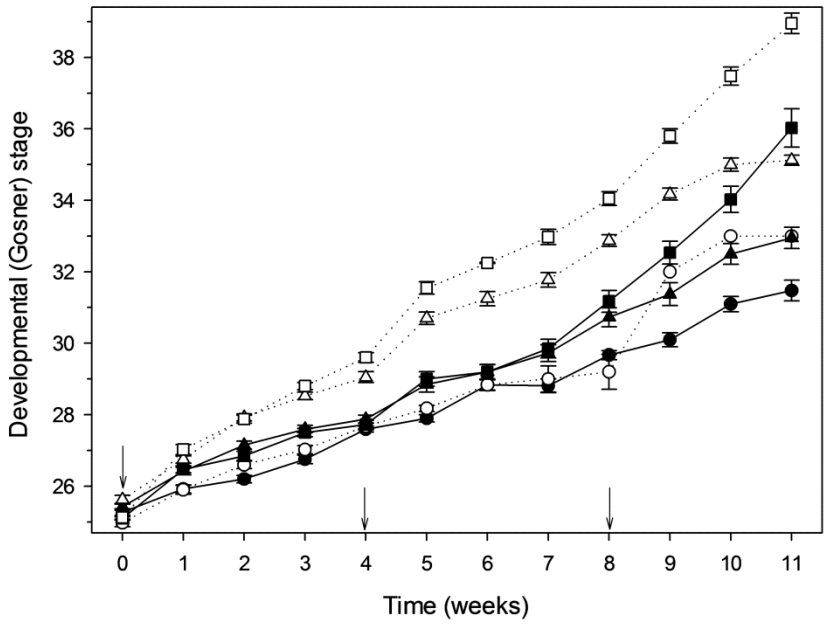

Developmental (Gosner) stage
26
28
30
32
34
36
38
0
1
2
3
4
5
6
7
8
9
10
11
Time (weeks)

Figure 5

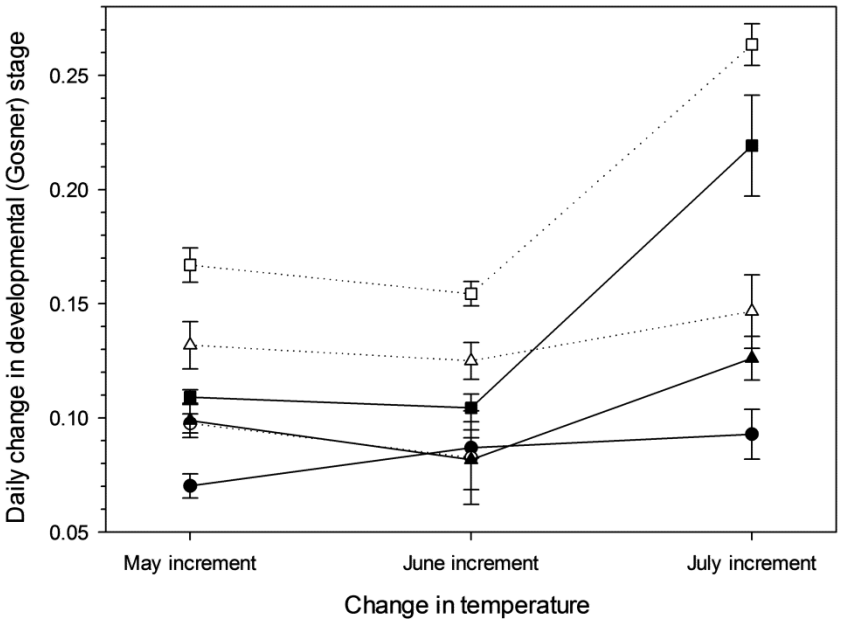
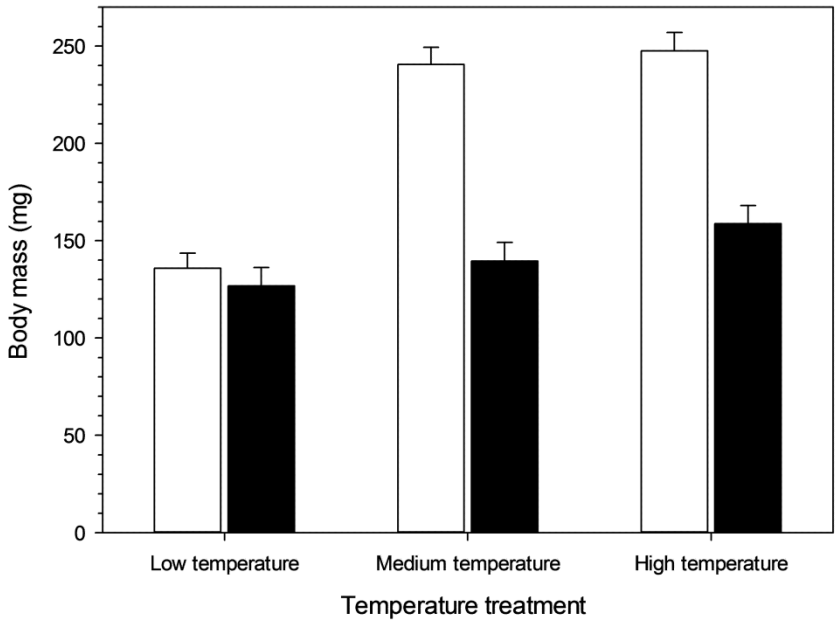

Daily change in developmental (Gosner) stage
0.25
0.20
0.15
0.10
0.05
May increment
June increment
July increment
Change in temperature

Figure 6


Body mass (mg)
250
200
150
100
50
0
Low temperature
Medium temperature
High temperature
Temperature treatment